AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning at page 9, line 22, with the following amended paragraph:,

Fig. 3: Short mRNA templates. The DNA primer-template pair used to synthesize the longest mRNA is illustrated at the top. The predicted translation products from our purified system are also shown (aminoacyl tRNAs for the 3' terminal codons GAA (Glu) and UUC (Phe) were not used). bK: biotin-labeled-lysine. S-D: Shine and Dalgarno ribosome binding site. The SEQ ID NOs for the nucleic acid and their respective amino acid sequences, where applicable, are designated as follows: T7 RNA pol, promoter (SEQ ID NO: 1); reverse complement of mRNA MTTV and T7 RNA pol promoter, (SEQ ID NO:2); mRNA MTTV (SEQ ID NOS: 3 and 10); mRNA MTV (SEQ ID NOS: 4 and 11); mRNA MV (SEQ ID NOS: 5 and 12); mRNA MVT (SEQ ID NOS: 6 and 13); mRNA scramble-epsiMVT (SEQ ID NOS: 7 and 13); mRNA ΔepsiMVT (SEQ ID NOS: 8 and 13); mRNA MTKV (SEQ ID NOS: 9 and 14).

Please replace the paragraph beginning at page 9, line 27, with the following amended paragraph:

Fig. 4: Characterization of oligopeptide synthesis rates from mRNA MTV in a purified his-tagged translation system. fMTV (SEQ ID NO: 11) was measured by ^{3}H-valine incorporated into peptide products in translations containing IF1H, IF2H, IF3H, EF-TuH, EF-GH and 0.020 A_{260}/μl ribosomes. *Triangles:* translations were started by mixing preincubated initiation components with preincubated elongation components. *Squares*: translations were started by transfering the translation mix from 0°C to 37°C. Aliquots were terminated with NaOH at the indicated times beginning at 1 min. Peptide product d.p.m. was calculated by subtracting d.p.m. obtained in aliquots terminated before 37°C incubation. Individual data points from representative experiments are plotted, with variations estimated to be less than 20%. A tangent line to the preincubation reaction curve is drawn to estimate the steady state rate.

Please replace the paragraph beginning at page10, line 40, with the following amended paragraph:

Fig. 9. Chemical biotinylation of Cys-tRNA^{Cys} (SEQ ID NO: 17).

Please replace the paragraph beginning at page 11, line 8, with the following amended paragraph:

Fig. 11. Incorporation of purified biotinyl-Cys into fM-T-bC-V (SEQ ID NO: 20) peptidomimetic using the purified system. Translations contained mMTCV, tRNAs charged with fM, T, V, and either uncharged, Cys-charged or purified biotinyl-Cys-charged tRNA, with controls lacking mRNAs. Selection was with Soft Avidin as in Fig. 8. Incomplete binding of ^{3}H-peptide with biotinyl-Cys substrate was likely due to the low affinity of Soft Avidin (an avidin derivatised to have a much higher K_d of approximately 10^{-7}M) for peptides containing a single biotin.

Please replace the paragraph beginning at page 11, lines 15, with the following amended paragraph:

Fig. 12. Assay for incorporation of adjacent large unnatural amino acids into fM-T-bC-bC-V peptidomimetic (SEQ ID NO: 21). The experiment was carried out as in Fig. 11. Note binding by Soft Avidin was complete for fM-T-bC-bC-V but incomplete for fM-T-bC-V, as expected for the much higher affinity for peptides containing more than one biotin SEQ ID NOS: 18 and 19 correspond to mMTCV and mMTCCV, respectively.

Please replace the paragraph beginning at page 11, lines 23, with the following amended paragraph:

Fig. 14. A generalizable approach for the synthesis of aminoacyl-tRNAs charged with unnatural amino acids specific for the codon(s) of choice. The elongator $tRNA^{Asn-CA}$, (SEQ ID NO: 24) synthesized *in vitro* (Fig. 16, lane 5) from our recombinant DNA clone prepared from synthetic oligodeoxyribonucleotides, contains substantial base alterations from the natural $tRNA^{Asn}$ sequence (SEQ ID NO: 22) that are indicated by arrows. The anticodon of the tRNA is indicated with large letters. An amino NVOC-protected unnatural amino acid was chemically aminoacylated on pdCA (SEQ ID NO: 33) (see upper right) and then ligated to the $tRNA^{Asn-CA}$ (produced by run-off transcription of Fok I cut template) with T4 RNA ligase (Fig. 16, lane 6). The approach is generalizable because no aminoacyl-tRNA-synthetase or natural tRNA was required.

Please replace the paragraph beginning at page 11, line 33, with the following amended pargraph:

Fig. 15. Three anticodon mutants of $tRNA^{Asn}$(N) termed, from left to right, $tRNA^{Asn}$(T) (SEQ ID NOS: 25 and 28) $tRNA^{Asn}$(S) (SEQ ID NOS: 26 and 29) $tRNA^{Asn}$(V) (SEQ ID NOS: 27 and 30). The new anticodons of the tRNAs are indicated with large letters above the codons

that they recognise. The genetic code has been redesigned so that the codons now specify whichever amino acid (natural or unnatural) is chosen to be ligated onto each $tRNA^{Asn-CA}$.

Please replace the paragraph beginning at page 12, line 16, with the following amended paragraph:

Fig. 19: Our spacer mRNAs (SEQ ID NO: 34) and their encoded polypeptide products: 13-mer (SEQ ID NO: 36), 21-mer (SEQ ID NO: 35), 41-mer (SEQ ID NO: 37), 53-mer (SEQ ID NO: 38, 61-mer (SEQ ID NO: 39, 101-mer (SEQ ID NO: 40).

Please replace the paragraph beginning at page 12, lines 22, with the following amended paragraph:

Fig. 21: Pure mRNA display. This is a pure version of the crude *in vitro* system (without living material) of Nemoto *et al.* (*supra*) and Roberts and Szostak (*supra*) based on that of Mattheakis *et al.* (Fig. 18) that differs in that the mRNAs are conjugated with puromycin (Pm) to enable covalent fusion of the mRNAs to their peptide or peptidomimetic products. Thus, the mRNA-peptidomimetic fusion may be purified from other translation components before selection, enabling very short peptidomimetics to be displayed without masking by the ribosome tunnel. Since the fusion reaction is slow, it is important to omit release factors when using mRNAs containing stop codons to prevent release factor-catalysed peptide release. Typically, ribosomes are stalled by a deoxynucleotide sequence conjugated to the mRNA. We ligated mMTKV and mMTCV (Figs. 8 and 11) to $pdA_{27}dCdCPm$ (SEQ ID NO: 41) (Trilink) efficiently using a custom-synthesized "splint" DNA (TTTTTTTTTTAATTCAAC, (SEQ ID NO: 46) designed to hybridize to the ends of either mRNA and also the $pdA_{27}dCdCPm$), and T4 DNA ligase, then gel-purified the conjugates for use in fusion and selection experiments (Roberts and Szostak (1997) *PNAS* 94, 12297-12302) where X in the figure is a readily selectable amino acid such as C or bC (Fig. 9) or bK (Fig. 22).

Please replace the paragraph beginning at page 16, line 18, with the following amended paragraph:

By a "start codon" is meant three bases which signal the beginning of a protein coding sequence. Generally, these bases are AUG (SEQ ID NO: 42) (or ATG); however, any other base triplet capable of being utilized in this manner may be substituted.

Please replace the paragraph beginning at page 22, line 7, with the following amended paragraph:

Tetrapeptide synthesis. We next investigated the suitability of this system for the synthesis of tetrapeptides to show that all steps of initiation and elongation could occur in a highly purified system. In contrast to tripeptide synthesis, tetrapeptide synthesis is not possible without dissociation of deacylated tRNA from the exit site of the ribosome(Wilson and Noller (1998) Cell 92, 337-49). Using a template encoding the tetrapeptide fMTTV (Fig. 3), synthesis of dual-labeled products was assessed by reversed phase HPLC (Fig. 6). Radioactive peptide products were identified based on their co-migration with chemically synthesized non-radioactive standards. The predominant radioactive peak in the peptidyl separation range corresponds to the fMTTV tetrapeptide (80-85% of the ^{14}C or ^{3}H radioactivity in this range) with a T/V ratio close to that expected. Two minor peaks correspond to the pausing or premature termination products fMT and fMTT, with no ^{3}H incorporated, as expected. fMT (SEQ ID NO: 15) and fMTT (SEQ ID NO: 16) together contain 12% of the ^{14}C radioactivity in the peptidyl range, equivalent to 20% of the combined products fMTTV, fMTT and fMT on a molar basis. The remaining two minor peaks (at 24 and 50 min.) presumably correspond to derivatives of fMTTV (*e.g.* methionine oxidation products or unformylated peptide), other peptidyl products and/or non-peptide radioactive contaminants. Thus, the purified his-tagged tetrapeptide translation system is predominantly, but not completely, processive with yields of full-length tetrapeptide products equal to 80% of the peptide products. This detailed analysis demonstrated much higher processivity in absence of added EF-P, W and rescue than that previouly reported (Ganoza et al. (1985) Proc Natl Acad Sci U S A 82, 1648-52).

Please replace the paragraph beginning at page 22, line 28, with the following amended paragraph:

Further examples of peptides synthesized efficiently by our *in vitro* translation system are shown in the Table 2. The synthesis of the expected 7-mer full-length fMTTTTTV peptide (SEQ ID NO: 44), generated from mRNA template product (SEQ ID NO. 43) was highly processive because a predominance of premature termination products would have resulted in a much higher T/V ratio than the measured value of 6.4.

Please replace the paragraph beginning at page 27, line 8, with the following amended paragraph:

We have tested incorporation of several unnatural amino acids using chemically charged tRNAs. The first step was to construct a synthetic elongator tRNA lacking the terminal CA dinucleotide to allow chemical misacylation with an unnatural amino acid in a generalisable manner. Current technology relies on artificial suppressor tRNAs that have been specially engineered to prevent charging and proofreading by any of the synthetases. In our pure system, our only concern was the possible effects of an expected lack of tRNA base-modifying activities because such modifications can be important for function (Bjork et al. (1999) FEBS Lett. 452,

47-51), and crude translation systems can modify synthetic tRNAs (Claesson et al. (1990) FEBS Lett. 273, 173-6). As new test prototypes, we chose *E. coli* $tRNA^{Asn}$ (Fig. 14; Ohashi *et al.* (1976) *Nucleic Acids Res.* 3, 3369-3376) and $tRNA^{Ala}$ (discussed below; Picking *et al.* (1991) *Nucleic Acids Res.* 19, 5749-5754). The 5' terminal sequence of $tRNA^{Asn}$ (SEQ ID NO: 23) was mutated for optimal transcription by T7 RNA polymerase, although alternative strategies to mutagenesis exist, such as the use of M1 RNA or RNase P to process synthetic unmodified tRNA precursors (Forster and Altman (1990) *Science* 249, 783-786). The anticodons of both tRNAs were also mutated to create several variants with altered codon recognition properties (three of our $tRNA^{Asn}$ mutants are shown in Fig. 15, with the amino acid codons recognised by the tRNAs indicated in brackets). An unnatural amino acid, allylglycine (aG, sometimes alternatively abbreviated 2P), was amino-protected with an NVOC group and ligated onto to the $tRNA^{Asn}$(N) (Fig. 14) using T4 RNA ligase in a standard and generalizable strategy (see Materials and Methods; Thorson *et al.* (1988) *Methods in Molecular Biology* 77, 43-73; Steward and Chamberlin (1998) *Methods in Molecular Biology* 77, 325-354) to give a species that migrated on a gel with the expected mobility (Fig. 16, lane 6).

Please replace the paragraph beginning at page 27, line 32, through page 28, line 9, with the following amended paragraph:

The amino group of the NVOCaG-$tRNA^{Asn}$(N) was deprotected by ultraviolet photolysis, and the aG-$tRNA^{Asn}$(N) added to a pure translation reaction containing mMTNV template (SEQ ID NO: 31). aG was successfully incorporated at the N codon to allow downstream ^{3}H-V incorporation, but gave a lower yield than the mMTV control translation (Fig. 17). The lower yield was found to be predominantly due to using insufficient aG-$tRNA^{Asn}$(N) to saturate the system, as doubling the aG-$tRNA^{Asn}$(N) RNA concentration to 1 μM (double the concentration of each natural aminoacyl tRNA in the translation, assuming 100% yield of aG-$tRNA^{Asn}$(N) from the transcription and ligation reactions) doubled the yield and incorporated the analog with about 65% processivity (based on incorporation of ^{14}C-T before the analog and ^{3}H-V after; result not shown). Translation of mMTNNV (SEQ ID NO: 32) demonstrated incorporation of adjacent analogs, with the % processivity for incorporation of the 2nd analog similar to the first under these analog substrate-limited conditions. Note that the translations of mMTNV and mMTNNV with an uncharged tRNA having the same structure as the $tRNA^{Asn}$(N) of Fig. 14, except ending with the normal ubiquitous CCA 3' sequence (produced by run-off transcription of BstNI cut template) gave no incorporation, demonstrating that the purified system lacked charging activity for this mutant $tRNA^{Asn}$(N), and was specific for incorporation of exogenous substrate at the N codon. As an additional control, uncharged $tRNA^{Asn}$(N) did not inhibit translation of mMTV (Fig. 17).

Please replace the paragraph beginning at page 35, line 11, with the following amended paragraph:

Construction of plasmids for the over-expression of his-tagged and untagged E. coli IF1, IF2 and IF3 proteins. *E. coli* initiation factor coding sequences, each containing an insertion of six histidines immediately after the N-terminal methionine, were synthesized by PCR from published plasmids and sub-cloned into a vector derived from pET24a (Novagen). Plasmid pXR201 containing the native IF1 sequence encoded by an artificial sequence of *E. coli*-preferred codons (instead of *infA* codons) was kindly supplied by R. Spurio and C. Gualerzi (Calogero et al. (1987) Mol Gen Genet 208, 63-9) and sub-cloned to give pAF1H. Plasmid pSL4 containing the native IF2 sequence encoded by *infB* was kindly supplied by S. Laalami and M. Grunberg-Manago (Laalami et al. (1991) J Mol Biol 220, 335-49) and sub-cloned to give pAF2H. Plasmid pDD1 containing the native IF3 sequence encoded by *infC* was kindly supplied by N. Brot and I. Schwartz (De Bellis and Schwartz (1990) Nucleic Acids Res 18, 1311) and sub-cloned to give pAF3H. The sequences of the three subclones, characterized by a combination of restriction digests and sequence analyses, begin (ligation sites underlined) with TATACA/TATG$(CAC)_6$ (SEQ ID NO: 47) before the second amino acid; the final amino acid is followed by the sequence TAAG/AATTCGAGCTCCGTCGA/42 bp deletion/AGATCC (SEQ ID NO: 48), and the remainder of the sequence is from pET24a. Analogous methods were also used to clone and over-express untagged versions of IF1, IF2 and IF3.

Please replace the paragraph beginning at page 41, line 4, with the following amended paragraph:

Measurement of processivity of synthesis of a 7-mer peptide (SEQ ID NO: 44). Peptide product d.p.m. from 5.1 µl coupled transcription/translations containing translation factors IF2, IF3, EF-Tu and EF-G are converted to pmoles.

Please replace the paragraph beginning at page 44, line 5, with the following amended paragraph:

Incorporation of two different types of unnatural amino acids into a peptidomimetic product. O-methyl serine is abbreviated as mS. Four artificial aminoacyl tRNAs were used: mS-tRNA(T), aG-tRNA(N), aG-tRNA(S), and aG-tRNA(V), with the respective mRNA codons recognised by each artificial tRNA given in parentheses. The highly labelled ^{3}H-amino acid was E. Total d.p.m.: total ^{3}H d.p.m. eluted from mini-column. All translations contained translation factors IF1, IF2, IF3, EF-Tu, and EF-G. The sequence of mRNA mMTNSVE is provided as SEQ ID NO: 45.